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Immunological studies on treponemal antigens *II. Serological changes and resistance to infection in rabbits immunized with culture supernatant of avirulent* Treponema pallidum

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In a previous paper, Izzat, Smith, Jackson, and Knox (1971) reported the isolation of an antigenic substance from the supernatant of cultures of avirulent *Treponema pallidum*. The antigenic substance was shown to be nontoxic and glycoprotein in nature. Injections of this material into rabbits stimulated precipitin antibodies reactive with FTA–ABS desiccated antigen.

The object of the present investigation was to evaluate the efficiency of the isolated antigenic substance in producing resistance to challenge with virulent *T. pallidum*.

Material and Methods

The virulent Nichols strain of *Treponema pallidum* obtained from Dr. G. R. Cannefax of the National Center of Disease Control, Atlanta, Georgia, was maintained in normal rabbit testes and was transferred successively every 2 weeks. This strain was used as the challenging agent throughout our study.

Adult New Zealand male rabbits, weighing 5 to 6 lb., which were seronegative to the VDRL test and showed no evidence of infection with *Treponema cuniculi*, were used throughout this study. They were housed in individual cages in a specially designed room that maintained environmental temperature at precisely 70°F.

IMMUNIZATION MATERIAL

Using the extraction method cited in our previous paper (Izzat, Smith, and others, 1971), the ammonium sulphate precipitated antigen was concentrated and lyophilized. At the time of immunization this lyophilized material was suspended in physiological saline to a final concentration of 25 mg./ml. and mixed with complete Freund's adjuvant (v/v). For the control antigen, Spirolate medium subjected to the same extraction method and lyophilization was re-suspended in physiological saline to the same final concentration.

IMMUNIZATION PROCEDURE

Two groups of male rabbits were used. In the first group, four rabbits were immunized subcutaneously with the supernatant antigen mixed with complete Freund's adjuvant (v/v). They were given increasing doses (5 mg., 25 mg., 50 mg., 100 mg., and 100 mg.) at weekly intervals for 5 weeks. The total amount given to each rabbit was thus 280 mg. lyophilized supernatant or 168 mg. antigenic protein. Seven control rabbits were inoculated as follows: two with a total of 280 mg. lyophilized medium plus serum and adjuvant; one with 280 mg. medium plus adjuvant without the rabbit serum supplement; one with complete Freund's adjuvant; one with rabbit serum. Material given to the two latter rabbits was based on volume of injection rather than weight. The remaining two control rabbits served as controls for the challenging organisms and received saline only.

In the second group, eleven rabbits were immunized subcutaneously for 24 weeks, using the supernatant antigen re-suspended in the same manner as described above. This material was administered weekly with increasing increments (from 5 to 100 mg.) to a total dosage of 1,300 mg. supernatant antigen per animal. In the same group, five control rabbits were inoculated subcutaneously as follows: two with 1,300 mg. of the lyophilized medium plus serum and adjuvant; and the remaining three rabbits with saline only to serve as controls for the challenging organisms.

INFECTIVITY TESTS

After completion of the immunization schedule, two of the test animals in Group 1 were challenged intradermally with 25×10^3 virulent *Treponema pallidum* cells suspended in 0·1 ml. of 50 per cent. rabbit serum in saline; each received four challenge injections on the skin of the lower back. The two remaining rabbits in this group, all the test rabbits of Group 2, and all the controls in both groups were challenged intradermally with 50 organisms per site. The challenging doses were determined according to the method of Izzat, Knox, Werth, and Dacres (1971). The animals were observed daily for the development of chancres. All lesions were subjected to darkfield examination.

SEROLOGICAL TESTS

The VDRL, FTA–ABS, and TPI tests for syphilis were performed weekly on all rabbits throughout the immunization period and after challenge. The Houston City Health Department Laboratory and the Venereal Disease Research Laboratory, National Center for Disease Control, Atlanta, Georgia, performed these tests.

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Results

Subcutaneous administration of 280 mg. supernatant antigen in complete Freund's adjuvant resulted in VDRL seroconversion in three of the four test animals before challenge (Table IA). This seroreactivity increased quantitatively after challenge with virulent *Treponema pallidum*. All sera were reactive in the FTA–ABS tests by the end of the challenge period while the TPI tests remained nonreactive. All seven control animals (Table IB) be came seropositive to the VDRL and FTA–ABS tests after challenge, but only one (Rabbit 1190) had shown seropositivity to the VDRL test before challenge. This rabbit had received 280 mg. medium plus adjuvant.

All test and control animals (Table IA, B) developed darkfield positive lesions demonstrating lack of resistance to the challenge organisms.

Immunization with higher doses of the supernatant antigen (1,300 mg. per rabbit) over a period of 24 weeks confirmed the above experimental data.

TABLE IA *Relationship between avirulent* T. pallidum *supernatant antigens and rabbit resistance in test animals*

Rabbit no.	*Initial VDRL*	*Serological test*	*Pre-challenge (5 wks)*	*Challenge dose per site*	*Post-challenge (wks)* 1	2	3	4	*Resistance to challenge*
1186	NR	VDRL	R : UND	25×10^3	R 1 : 8	R 1 : 8	R 1 : 8	R 1 : 32	Developed darkfield positive lesions at all sites within 14 days after challenge
		FTA–ABS	NR		NR	NR	R	R	
		TPI	NR		NR	NR	NR	NR	
1187	NR	VDRL	R : UND	25×10^3	WR	R : UND	R 1 : 4	R 1 : 8	
		FTA–ABS	NR		NR	NR	R	R	
		TPI	NR		NR	NR	NR	NR	
1188	NR	VDRL	R : UND	50	WR	WR	WR	R 1 : 2	Developed darkfield positive lesions at all sites within 29 days after challenge
		FTA–ABS	NR		NR	NR	NR	R	
		TPI	NR		NR	NR	NR	NR	
189	NR	VDRL	NR	50	NR	NR	WR	R : UND	
		FTA–ABS	NR		NR	NR	NR	R	
		TPI	NR		NR	NR	NR	NR	

NR—Nonreactive WR—Weakly reactive R—Reactive R : UND—Reactive undiluted

TABLE IB *Relationship between avirulent* T. pallidum *supernatant antigens and rabbit resistance in control rabbits*

Rabbit no.	*Immunization material*	*Initial VDRL*	*Serological test*	*Pre-challenge (5 weeks)*	*Post-challenge (wks)* 1	2	3	4	*Resistance to challenge*
1190	Lyophilized medium + serum with adjuvant	NR	VDRL	R : UND	R 1 : 2	R 1 : 8	R 1 : 4	R 1 : 8	
			FTA–ABS	NR	NR	NR	R	R	
			TPI	NR	NR	NR	NR	NR	
1191	Lyophilized medium + serum with adjuvant	NR	VDRL	NR	WR	WR	WR	R 1 : 2	
			FTA–ABS	NR	NR	NR	R	R	
			TPI	NR	NR	NR	NR	NR	
1192	Lyophilized medium with adjuvant	NR	VDRL	NR	NR	NR	NR	R : UND	All animals developed darkfield positive lesions at all sites within 29 days post challenge with 5×10 organisms/site
			FTA–ABS	NR	NR	NR	NR	R	
			TPI	NR	NR	NR	NR	NR	
1193	Rabbit serum only	NR	VDRL	NR	NR	NR	R 1 : 4	R : Und	
			FTA–ABS	NR	NR	NR	R	R	
			TPI	NR	NR	NR	NR	NR	
1194	Adjuvant only	NR	VDRL	NR	NR	NR	WR	R 1 : 2	
			FTA–ABS	NR	NR	NR	R	R	
			TPI	NR	NR	NR	NR	NR	
1195	None	NR	VDRL	—	NR	NR	WR	R 1 : 4	
			FTA–ABS		NR	NR	R	R	
			TPI		NR	NR	NR	NR	
1196	None	NR	VDRL	—	NR	NR	R 1 : 2	R 1 : 2	
			FTA–ABS		NR	NR	R	R	
			TPI		NR	NR	NR	NR	

NR—Nonreactive WR—Weakly reactive R—Reactive R : UND—Reactive undiluted

Nine of the eleven test animals demonstrated VDRL seroconversion. Simultaneously the two controls (Table IIA, B) that had received medium plus adjuvant developed reactivity to the VDRL test. Sera from all the test animals were reactive in the FTA-ABS test before challenge, but none of the sera from control animals showed such reactivity. After challenge with virulent *T. pallidum,* darkfield

TABLE IIA *Resistance and serological changes of test rabbits immunized subcutaneously with lyophilized supernatant and challenged with virulent* Treponema pallidum.

Rabbit no.	*Initial VDRL*	*Serological tests*	*Pre-challenge (wks)* 4	10	14	20	24	*Post-challenge (wks)* 2	6	10	*Resistance to (5 × 10 org./site)*
1197	NR	VDRL	NR	NR	NR	NR	NR	NR	R : UND	R 1 : 2	All animals developed dark field positive lesions at all sites within 29 days post challenge
		FTA-ABS	NR	NR	NR	R	R	R	R	R	
1198	NR	VDRL	NR	WR	R 1 : 8	R 1 : 8	R 1 : 32	R 1 : 32	R 1 : 32	R 1 : 64	
		FTA-ABS	NR	R	R	R	R	R	R	R	
1199	NR	VDRL	NR	WR	WR	R : UND	R : UND	R : UND	R 1 : 32	R 1 : 32	
		FTA-ABS	NR	R	R	R	R	R	R	R	
1200	NR	VDRL	WR	WR	WR	R 1 : 2	R 1 : 2	R 1 : 4	R 1 : 8	R 1 : 64	
		FTA-ABS	NR	R	R	R	R	R	R	R	
1201	NR	VDRL	NR	NR	NR	NR	NR	NR	R : UND	R 1 : 32	
		FTA-ABS	NR	R	R	R	R	R	R	R	
1202	NR	VDRL	R : UND	R : UND	R 1 : 2	WR	NR	NR	R 1 : 4	R 1 : 16	
		FTA-ABS	NR	R	R	R	R	R	R	R	
1203	NR	VDRL	R 1 : 2	R : UND	R 1 : 4	R 1 : 2	R 1 : 2	R 1 : 2	R 1 : 16	R 1 : 32	
		FTA-ABS	NR	R	R	R	R	R	R	R	
1204	NR	VDRL	R 1 : 2	R 1 : 2	R 1 : 2	WR	WR	WR	WR	WR	
		FTA-ABS	NR	R	R	R	R	R	R	R	
1205	NR	VDRL	WR	R 1 : 2	R 1 : 2	R 1 : 2	R 1 : 2		Died		
		FTA-ABS	NR	R	R	R	R				
1206	NR	VDRL	NR	R : UND	NR	NR	NR	WR	R 1 : 8	R 1 : 32	
		FTA-ABS	NR	R	R	R	R	R	R	R	
1207	NR	VDRL	NR	R 1 : 2	R 1 : 16	R 1 : 32	R 1 : 32	R 1 : 64	R 1 : 64	R 1 : 128	
		FTA-ABS	NR	R	R	R	R	R	R	R	

TABLE IIB *Resistance and serological changes of control rabbits immunized subcutaneously with lyophilized supernatant and challenged with virulent* Treponema pallidum

Rabbit no.	*Immunization material*	*Serological tests*	*Pre-challenge (wks)* 4	10	14	20	24	*Post-challenge (wks)* 2	6	10	*Resistance to challenge (5 × 10 org./site)*
1208	Lyophilized medium + serum with adjuvant	VDRL	NR	R : UND	R : UND	R 1 : 2	R : UND	R 1 : 2	R : UND	R 1 : 128	All animals developed dark field positive lesions at all sites within 29 days post challenge
		FTA-ABS	NR	NR	NR	NR	NR	NR	R	R	
1209	Lyophilized medium + serum with adjuvant	VDRL	R : UND	R 1 : 2	R 1 : 8	R 1 : 16	R 1 : 32	R 1 : 32	R 1 : 32	R 1 : 128	
		FTA-ABS	NR	NR	NR	NR	NR	R	R	R	
1210	None	VDRL	NR	NR	NR	NR	NR	NR	R 1 : 16	R 1 : 16	
		FTA-ABS	NR	NR	NR	NR	NR	R	R	R	
1211	None	VDRL	NR	NR	NR	NR	NR	NR	R 1 : 2	R 1 : 16	
		FTA-ABS	NR	NR	NR	NR	NR	NR	R	R	
1212	None	VDRL	NR	NR	NR	NR	NR	NR	WR	R 1 : 16	
		FTA-ABS	NR	NR	NR	NR	NR	NR	R	R	

NR—Nonreactive WR—Weakly reactive R—Reactive R : UND—Reactive undiluted

positive lesions appeared at the same time (24 to 29 days) in both test and control rabbits. The VDRL titres were maintained at essentially the same level for 2 weeks after challenge, and then gradually increased throughout the remainder of the challenge period (Table IIA, B). The FTA–ABS tests were also reactive throughout this period.

Discussion

The antigenic complex isolated from the culture supernatant of avirulent *T. pallidum* is immunogenic. Animals inoculated subcutaneously with the supernatant antigen complex produced antibodies reactive in the VDRL and FTA–ABS tests. This finding is an agreement with that of Deacon and Hunter (1962), Király, Jobbágy and Kováts (1967), Meyer and Hunter (1967), and Tringali and Cox (1970), who have reported the existence of common antigens between cultivatable and pathogenic treponemes. The fact that the VDRL antibodies were induced both in animals immunized with the supernatant and in animals immunized with the medium suggests the presence of a nonspecific antigen in the medium. This hypothesis is also suggested by the related findings of Cannefax, Hanson and Skaggs (1968), Rathlev (1968), and Wilkinson and Ferguson (1968), who have demonstrated that several components of sorbents and uninoculated Reiter culture medium are able to remove nonspecific reactivity from non-syphilitic human sera.

Results obtained by the FTA–ABS test on rabbits immunized with supernatant are of particular interest. All animals immunized with the supernatant material produced antibodies reactive in the specific FTA–ABS test. This reactivity indicates the presence of an antigen in the supernatant complex responsible for the induction of specific antibody *in vivo*. The reactivity also corresponds with the precipitin reaction between rabbit antiserum and the FTA–ABS antigen observed in immunoelectrophoresis (Izzat, Smith, and and others, 1971).

The results of serological tests on the rabbit sera after challenge failed to establish any relationship to resistance, since animals immunized with medium demonstrated higher VDRL titres than those immunized with supernatent antigen. Although the FTA–ABS test was reactive in both test and control animals after challenge, syphilitic chancres developed in both test animals and nonimmunized controls at approximately the same time. These findings as well as those of our previously reported study (Izzat, Dacres, Knox, and Wende, 1970) support the belief of Magnuson, Thompson, and McLeod (1951) and Miller, Whang, and Fazzan (1963) that circulating antibodies may play little or no role in immunity against syphilis.

Summary

Rabbit hyperimmune antisera experimentally produced against culture supernatant of avirulent *T. pallidum* were reactive in the VDRL and FTA–ABS tests. The VDRL reactivity was induced in both test and control animals, while the FTA–ABS reactivity was demonstrated only in the test animals. Hyperimmunization with the supernatant antigen did not protect animals against challenge doses of virulent *T. pallidum*.

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Études immunologiques sur les antigènes tréponémiques
II. Modifications sérologiques et résistance à l'infection de lapins immunisés par injections de surnageat de cultures de *Treponema pallidum* non virulent

SOMMAIRE

Des anti-sérums de lapins hyperimmunisés expérimentalement avec le surnageat de cultures de *T. pallidum* non virulent se montrèrent réactifs dans les épreuves de VDRL et de FTA–ABS. La positivité au VDRL fut constatée à la fois chez les animaux considérés et chez les témoins alors que la positivité pour le FTA–ABS ne fut trouvée que pour les animaux hyperimmunisés. L'hyperimmunisation avec l'antigène du surnageat ne protégea pas les animaux contre l'inoculation de *T. pallidum*.